PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

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PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Rule 71.1)

Date of mailing *(day/month/year)*	01.02.2005
Applicant's or agent's file reference SCB828PCT	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP 03/14719	22.12.2003	23.12.2002

Applicant
ISTITUTO NAZIONALE PER LO STUDIO E LA CURA.. et al

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

The applicant's attention is drawn to Article 33(5), which provides that the criteria of novelty, inventive step and industrial applicability described in Article 33(2) to (4) merely serve the purposes of international preliminary examination and that "any Contracting State may apply additional or different criteria for the purposes of deciding whether, in that State, the claimed inventions is patentable or not" (see also Article 27(5)). Such additional criteria may relate, for example, to exemptions from patentability, requirements for enabling disclosure, clarity and support for the claims.

Name and mailing address of the international preliminary examining authority:	Authorized Officer
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Enclosure 3

CLAIMS

1. A method for determining the concentration of circulating total DNA in a plasma sample from a cancer patient, a subject with cancer susceptibility or at risk of developing cancer, which comprises:

1) extracting the DNA from the plasma sample;

2) adding to the DNA preparation: a) a mixture of oligonucleotide primers suitable for PCR amplification of a fragment of the human telomerase reverse transcriptase (hTERT) gene, and b) an oligonucleotide probe, having at least one quencher and one reporter fluorophore at the 3' and 5' ends, able to anneal to a sequence within the region delimited by the primers,
in suitable conditions for carrying out a PCR reaction,

3) adding a heat-stable DNA polymerase with 5'-3' hexonuclease activity and amplifying the hTERT gene fragment;

4) measuring the produced fluorescence.

2. A method according to claim 1, wherein the DNA concentration in the test sample is determined by interpolation of a calibration curve calculated with known amounts of DNA.

3. A method as claimed in claims 1-2, which further comprises comparing the concentration of circulating DNA to a reference concentration.

4. A method according to claim 3, wherein the reference concentration is from 9 to 25 ng/ml.

5. A method as claimed in claim 1, wherein said fragment of the human telomerase reverse transcriptase (hTERT) gene is from nt 13059 to nt 13156 of the sequence GenBank accession n. AF128893.

6. A method according to claim 5, wherein said fragment of the human telomerase reverse transcriptase (hTERT) gene is amplified using SEQ ID N.